

Review

The ubiquitin/26S proteasome system in plant–pathogen interactions: a never-ending hide-and-seek game

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SUMMARY

The ubiquitin/26S proteasome system (UPS) plays a central role in plant protein degradation. Over the past few years, the importance of this pathway in plant–pathogen interactions has been increasingly highlighted. UPS is involved in almost every step of the defence mechanisms in plants, regardless of the type of pathogen. In addition to its proteolytic activities, UPS, through its 20S RNase activity, may be part of a still unknown antiviral defence pathway. Strikingly, UPS is not only a weapon used by plants to defend themselves, but also a target for some pathogens that have evolved mechanisms to inhibit and/or use this system for their own purposes. This article attempts to summarize the current knowledge on UPS involvement in plant–microbe interactions, a complex scheme that illustrates the never-ending arms race between hosts and microbes.

INTRODUCTION

All cell processes, from division to death, include essential protein degradation steps. In eukaryotes, most of the protein degradation events are controlled by the ubiquitin/26S proteasome system (UPS) (Dreher & Callis, 2007). As sessile organisms that face environmental variations, plants have evolved a number of mechanisms to protect themselves against abiotic and biotic stresses, such as preformed physical barriers, antimicrobial compounds, and the perception and recognition of pathogen-associated molecular patterns (PAMPs) or pathogen race-specific effectors. Proteomic plasticity is a crucial element in these plant responses to abiotic and biotic stresses. UPS therefore plays a central role in plant defence by altering the proteome and thus increasing the chances of survival (Dreher & Callis, 2007). UPS involvement in plant defence mechanisms

occurs at different levels, from ubiquitin to 26S proteasome. Changes in ubiquitin and E1 and/or E2 enzyme levels may have broad effects on cell reprogramming during plant defence, and many research groups have shown recently that E3 ubiquitin ligases, the key components of UPS targeting specificity, are implicated in plant–pathogen interactions, including early defence reactions, gene-for-gene interactions and induced disease resistance (Delauré *et al.*, 2008; Zeng *et al.*, 2006).

This article attempts to place this new information in perspective, highlighting the crucial role played by UPS in plant disease responses, involving both the ubiquitin conjugating system and 26S proteasome. It also shows that pathogens are able to hijack this pathway, to counteract plant defence or to utilize host UPS components to their own advantage, revealing a complex UPS–pathogen interaction scheme.

UBIQUITIN/26S PROTEASOME: A POWERFUL SYSTEM TO REGULATE PROTEIN STABILITY

Ubiquitin conjugation pathway

Most UPS substrate proteins are covalently bound to ubiquitin, a 76-amino-acid protein, by a three-step energy-dependent mechanism prior to degradation by 26S proteasome (Fig. 1A). Ubiquitin is activated for transfer by the formation of a thiolester bond with a ubiquitin-activating enzyme (E1). Activated ubiquitin is then transferred to a ubiquitin-conjugating enzyme (E2), again through a thiolester bond. Finally, a ubiquitin ligase enzyme (E3) recruits and transfers ubiquitin to the target protein. The ubiquitin C-terminal glycine residue is linked to a substrate amino acid (generally a lysine residue) via an isopeptide bond (Vierstra, 2009). This mechanism is repeated to obtain polyubiquitinated target proteins (Dreher & Callis, 2007). The shortest polyubiquitin chain capable of activating proteasomal degradation is four monomers long (Thrower *et al.*, 2000), although many substrate proteins appear to be tagged with longer chains (Hanna & Finley, 2007).

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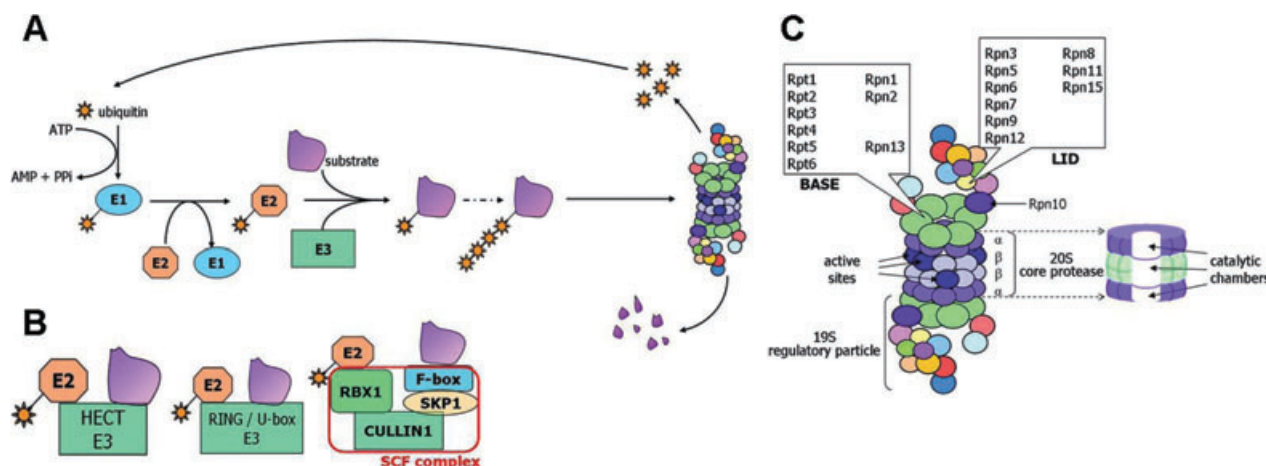


Fig. 1 An overview of the ubiquitin/26S proteasome system (UPS). (A) The UPS pathway begins with the ATP-dependent activation of ubiquitin by an E1 enzyme. Ubiquitin is then transferred to an E2 enzyme, and finally attached to the target protein via an E3 enzyme. Multiple cycles of ubiquitin conjugation lead to a polyubiquitinated substrate that is degraded by the 26S proteasome complex, releasing short peptides. (B) Types of E3 ubiquitin ligase enzymes. See text for details. (C) Structure of the 26S proteasome. The 20S core protease is composed of four heptameric rings, forming three cavities. The outer rings contain α subunits and the inner rings β subunits. Three of the β subunits (namely $\beta 1$, $\beta 2$ and $\beta 5$) harbour a protease activity. One or two 19S regulatory particles can be attached to the outer rings. The 19S regulatory particle is composed of two complexes, the lid and the base. The base contains six proteasomal ATPases attached to the α rings of the 20S proteasome. The Rpn10 subunit can interact either with the lid or the base and stabilizes the complex. Adapted from Delaure *et al.*, 2008.

The major role of polyubiquitination is to address the substrate protein to the 26S proteasome, where its peptide bonds are hydrolysed, reducing a folded protein into oligopeptides that are released in the cytoplasm (Hanna & Finley, 2007). However, ubiquitination not only targets proteins for degradation, but is involved in the regulation of post-translational modifications. The impact of ubiquitination depends on many factors, including the ubiquitin chain length and the particular amino acid of the ubiquitin chain through which it is attached to the target protein. Thus, proteins containing lysine-48 (Lys-48)-linked polyubiquitin tags are targeted for proteasomal degradation. In contrast, monoubiquitinated or polyubiquitinated proteins linked via ubiquitin Lys-63 are not degraded but involved in nonproteolytic events, such as subcellular localization or functional post-translational modification, protein activation or protein–protein interactions (Deng *et al.*, 2000; Manzano *et al.*, 2008; Schnell & Hicke, 2003). Notably, it has also been demonstrated that some proteins can be addressed to the 26S proteasome in a ubiquitin-independent manner, such as calmodulin or p53 (Orlowski & Wilk, 2003), but the associated mechanism(s) still remains unknown.

Different types of E3 ubiquitin ligase

The E3s, responsible for the final tagging of proteins, provide specificity to the UPS process. Large E3 ligase families are encoded in plant genomes, each family member controlling the ligation of ubiquitin to only one or a small subset of substrate

proteins, implying that a wide variety of targets may be recognized (Vierstra, 2003). Over 6% of the predicted *Arabidopsis thaliana* genome encodes UPS proteins (Dreher & Callis, 2007), including only two E1, 37 predicted E2 (Downes & Vierstra, 2005) and at least 1400 predicted E3 proteins. Plant E3s fall into different families on the basis of their subunit composition and mode of action (Lechner *et al.*, 2006). These families can be classified into two major groups, which covalently [HECT (homology to E6-associated protein C-terminus) E3s] and noncovalently [U-box domain and RING (really interesting new gene) E3s] bind to ubiquitin (Fig. 1B). HECT E3 ligases establish a covalent bond with ubiquitin before its transfer to the target protein (Dreher & Callis, 2007). To date, seven HECT E3s have been identified in the *A. thaliana* genome, among which UPL3 (HECT-containing ubiquitin protein ligase 3) is involved in trichome shape control (Downes *et al.*, 2003). U-box and RING E3s are thought to be structurally related and functionally similar, using hydrogen bonds/salt bridges or zinc chelation, respectively, to transfer ubiquitin to the substrate (Downes & Vierstra, 2005; Stone *et al.*, 2005). About 61 U-box domain E3s are predicted in the *A. thaliana* genome, but at least 475 proteins (about 2% of the predicted *A. thaliana* proteins) contain one or more RING motifs (Stone *et al.*, 2005, 2006). Two types of RING-type E3 ubiquitin ligase exist: single subunit E3 and RING-finger proteins as subunits of multiprotein E3 complexes. One of the most conserved multisubunit RING E3 families in eukaryotes is the cullin RING ligases (CRL), among which the modular SCF group is the largest and best characterized because of its roles in many cellular

processes. *Arabidopsis thaliana* SCF complexes are composed of four major subunits: cullin-1 (CUL1), SKP1 (S-phase kinase-associated protein)-like protein (ASK1/2), RBX1 (RING box protein) and F-box protein (Dreher & Callis, 2007). CUL1 acts as a scaffold in assembling the different subunits of the SCF complex by interacting at its C-terminal region with RBX1 and at its N-terminus with SKP1. RBX1 and SKP1 are linked to E2-ubiquitin and F-box protein, respectively. The RBX1–E2 association mediates the ubiquitin transferase activity, and the SKP1–F-box protein complex confers substrate specificity (Lechner *et al.*, 2006). The *A. thaliana* genome encodes about 700 F-box proteins (Devoto *et al.*, 2003; Gagne *et al.*, 2002), and 687 potential F-box proteins have been identified in *Oryza sativa* (Jain *et al.*, 2007), suggesting a high targeting potential. Interestingly, the number of F-box proteins in plants is significantly higher than in other eukaryotes, but the reason for such an expansion remains unclear to date (Thomann *et al.*, 2005).

The 26S proteasome

The structure of the 26S proteasome is highly conserved in eukaryotes and can be divided into two distinct particles: the 20S core proteasome (CP) and the 19S regulatory particle (RP) (Fig. 1C) (Vierstra, 2003). The CP is a broad-spectrum, ATP- and ubiquitin-independent protease, composed of four stacked rings, defining a barrel-shaped structure. The outer rings are composed of seven α subunits and the inner rings are composed of seven β subunits, which give the 20S complex the general structure of $\alpha_1\text{--}\beta_1\text{--}\beta_1\text{--}\alpha_1\text{--}\beta_1\text{--}\beta_1\text{--}\alpha_1\text{--}\beta_1$. Three proteolytic activities, carried by the β_1 , β_2 and β_5 subunits, are housed by the inner rings, defining a catalytic chamber. Access to this chamber is controlled by the α rings, which only allow unfolded proteins to enter (Kurepa & Smalle, 2007; Lorentzen & Conti, 2006; Vierstra, 2003). One or both CP outer rings can be capped by a 19S RP, composed of two complexes, the lid and the base (Kurepa & Smalle, 2007). The base contains three non-ATPase subunits (RPN) and a ring of six ATPase subunits (RPT) that interacts with the CP α subunits, and is probably involved in target unfolding and gate opening via ATP-dependent mechanisms. The lid, which binds to the base, generally contains nine RPN subunits, although some other subunits may also bind to the lid subcomplex in regulating 26S proteasome activity (Glickman & Raveh, 2005). Little is known about the lid subunit functions, except for RPN11, which has a deubiquitinating activity, and RPN5, 6 and 7, which are essential for subparticle assembly (Vierstra, 2003).

Interestingly, it has been demonstrated *in vitro* that animal and plant 20S proteasomes also harbour RNase activity. This activity was first observed with chicken liver proteasomes that degrade 18S rRNA *in vitro* (Tsukahara *et al.*, 1989). The authors showed that this activity was inactivated by heat treatment and by the addition of low sodium dodecylsulphate concentrations,

thus excluding the hypothesis of a contamination by low-molecular-mass RNases. Using *Tobacco mosaic virus* (TMV, *Tobamovirus*) RNA as a substrate, it has been demonstrated further that calf liver 20S proteasome RNase activity is an endonuclease activity, associated with the α_5 subunit (named zeta), and that this activity is not influenced by 20S integrity, as strong dissociating conditions (6 M urea) do not impair RNA degradation (Petit *et al.*, 1997; Pouch *et al.*, 1995). Petit *et al.* (1997) also showed that a lower endonuclease activity is associated with the α_1 subunit (named iota), but, to date, this observation has not been confirmed. Some RNAs containing tRNA-like structures, such as the *Human immunodeficiency virus* (HIV, *Retrovirus*) TAR (transactivation response) RNA, are also degraded (Gautier-Bert *et al.*, 2003). Furthermore, 20S RNA complex reconstruction *in vitro* and RNA residual fragment identification in purified proteasome fractions have confirmed that the animal 20S proteasome harbours an RNA endonuclease activity (Gautier-Bert *et al.*, 2003). Recently, the existence of a similar 20S proteasome endonuclease activity has also been demonstrated in purified sunflower proteasome, using TMV and *Lettuce mosaic virus* (LMV, *Potyvirus*) RNA substrates (Ballut *et al.*, 2003), indicating that the existence of such an activity is not specific to animal proteasomes.

THE UBIQUITIN/PROTEASOME PATHWAY IN PLANT DEFENCE REACTIONS

UPS plays a central role in the cell and targets two major types of protein: misfolded/damaged proteins and functional proteins carrying specific destruction signals. Therefore, UPS acts both as a quality control and regulatory system (Goldberg, 2003; Kurepa & Smalle, 2007). As a regulatory system, UPS is involved in mechanisms as essential as cell cycle control (Jurado *et al.*, 2008; Hershko, 2005; del Pozo *et al.*, 2006), programmed cell death (PCD) (Endo *et al.*, 2001; Jin *et al.*, 2006; Stone & Callis, 2007) and plant development (Brukhin *et al.*, 2005; Downes *et al.*, 2003; Kevany *et al.*, 2007; Schwager *et al.*, 2007; Sheng *et al.*, 2006), and is also implicated in self-incompatibility (Hua & Kao, 2008) and signal transduction cascades following light (Hoecker, 2005; Moon *et al.*, 2007), sucrose (Ellis *et al.*, 2002) or hormone (Bostick *et al.*, 2004; Manzano *et al.*, 2008; Stone *et al.*, 2006) signalling. Thus, UPS plays a role in plant responses to the majority of external environment changes. For example, a hot pepper U-box ubiquitin ligase mRNA is rapidly and highly induced in response to various environmental stress factors, including dehydration, high salinity and cold temperature (Cho *et al.*, 2006), proteasomal function is required to trigger PCD in heat-shocked plants (Vacca *et al.*, 2007) and 20S proteasome is involved in heavy metal resistance in cadmium- and nickel-stressed maize plants by scavenging metal-oxidized proteins (Forzani *et al.*, 2002; Pena *et al.*, 2007).

Plants face biotic stress in the form of insects, nematodes, fungi, bacteria or virus attacks on a daily basis. However, most plants are resistant to most pathogens because of the complex mechanisms they have developed to inhibit or limit damage. Plant defence can, in particular, be mediated by specific interactions between pathogen avirulence (*Avr*) and plant resistance (*R*) genes, triggering the deployment of active defence mechanisms and the containment of pathogens in restricted areas (Dangl & Jones, 2001). During the past few years, a growing body of evidence has indicated that UPS is not only implicated in crucial cellular surveillance mechanisms, but also that it can be involved in the defence of plants against pathogens. UPS appears to be required in various steps of the jasmonate, salicylic acid and ethylene signalling pathways (Binder *et al.*, 2007; Devoto *et al.*, 2002; Thines *et al.*, 2007; Yaeno & Iba, 2008), all three of which are involved in defence reactions against bioaggressors. Nevertheless, hormone signalling does not seem to be the only way in which UPS is involved in plant defence mechanisms. Indeed, UPS is an emerging actor in plant–microbe interactions.

Role of ubiquitination in plant defence

About 20% of *A. thaliana* gene expression changes under pathogen attack (Rietz & Parker, 2007) and the up-regulation of general components of UPS have commonly been observed in various plant–pathogen interactions. Protein (poly)ubiquitination may play a role in basal host resistance: in powdery mildew (*Blumeria graminis*)-attacked barley epidermis, depletion of cellular ubiquitin by transient-induced gene silencing (TIGS), contrary to the targeting of the 19S RP by RNA interference (RNAi), induces high susceptibility towards the compatible strain *B. graminis* f. sp. *hordei* (Dong *et al.*, 2006). The ubiquitin conjugation pathway is thus necessary for the enhanced protein degradation observed, but the 26S proteasome is not (Dong *et al.*, 2006). Ubiquitination may also be involved in gene-for-gene resistance: sunflower hypocotyls accumulate high levels of ubiquitin transcripts when challenged with an incompatible strain of powdery mildew (*Plasmopara halstedii*), but not with a compatible strain (Mazeyrat *et al.*, 1999). Furthermore, in *A. thaliana*, the *mos5* (modifier of *snc1* 5) suppressor of the gain-of-function *snc1* (suppressor of *npr1-1* constitutive 1) mutant has been shown to be affected in UPS (Goritschnig *et al.*, 2007). The *snc1* mutant corresponds to a point mutation in an *R* gene, resulting in the constitutive activation of defence responses without interaction with pathogens. Interestingly, the *mos5* mutant carries a 15-bp deletion in *AtUBA1* (ubiquitin-activating enzyme 1) (Goritschnig *et al.*, 2007), which is thus able to block the signalling cascade downstream of an *R* gene, through an as yet unknown mechanism. In fact, the *mos5* mutation only affects the resistance response conferred by a small subset of *R* proteins, suggesting that the ubiquitination pathway is essential for the

activation and downstream signalling of some, but possibly not all, *R* gene-mediated responses (Goritschnig *et al.*, 2007). UPS also appears to be implicated in compatible interactions. The altered response to viral infection of tobacco plants perturbed in UPS (Becker *et al.*, 1993), and the up-regulation of two tobacco ubiquitin-activating enzymes (NtE1A and NtE1B) by infection with *Tomato mosaic virus* (ToMV, *Tobamovirus*) and TMV (Takizawa *et al.*, 2005), support the idea that UPS participates in the molecular dialogue between viruses and compatible host plants.

E3 ubiquitin ligases mediating plant defence signalling

SGT1, an E3 ubiquitin ligase ubiquitous in plant defence

The first clue indicating that E3-mediated proteolysis contributes to *R* gene defence was provided by the rapid turnover of *A. thaliana* RPM1 (resistance to *Pseudomonas syringae* pv. *maculicola* 1), a nucleotide binding site leucine-rich repeat (NBS-LRR) *R* protein, coincident with the onset of the hypersensitive response (HR) (Boyce *et al.*, 1998). As RPM1 confers resistance to some *P. syringae* strains by triggering an HR, the authors suggested that this observation could correspond to a negative feedback loop used by the plant to control HR lesion size and response amplitude at the site of infection (Boyce *et al.*, 1998). The disappearance of RPM1 seems to be mediated by UPS as it is suppressed after treatment with a proteasome inhibitor (Kawasaki *et al.*, 2005). To date, the two identified RING-finger E3 proteins, RIN2 (RPM1-interacting protein 2) and RIN3, which interact directly with RPM1, are not required for its degradation, which suggests that other unidentified partners are involved (Kawasaki *et al.*, 2005) (Fig. 2A). Interestingly, it has been reported that RPM1 is undetectable in *A. thaliana rar1* (required for Mla12 resistance 1) mutants, suggesting that RAR1 regulates RPM1 stability (Tornero *et al.*, 2002). *RAR1* encodes a predicted cytosolic protein of unknown function, conserved in all eukaryotes except yeast (Shirasu *et al.*, 1999). It seems that RAR1 associates in *A. thaliana* with cytosolic HSP90 to act as a co-chaperone and to stabilize some NBS-LRR resistance proteins, such as RPM1 (Holt *et al.*, 2005; Hubert *et al.*, 2003). A yeast two-hybrid screen has shown that the *A. thaliana* orthologue of RAR1 interacts with AtSGT1 (suppressor of G2 allele of *skp1*), a plant orthologue of the yeast E3 protein SGT1, involved in cell cycle control, which associates with the SKP1 and cullin subunits of the SCF complex (Azevedo *et al.*, 2002). It has been shown by mutational analysis that AtSGT1b is required for *A. thaliana* resistance against *Peronospora parasitica* (Austin *et al.*, 2002; Tör *et al.*, 2002).

The involvement of RAR1 and SGT1 in defence mechanisms has also been highlighted in several other plants. In barley, RAR1 is required for powdery mildew resistance triggered by the Mla6 and Mla12 *R* proteins (Shirasu *et al.*, 1999). Indeed,

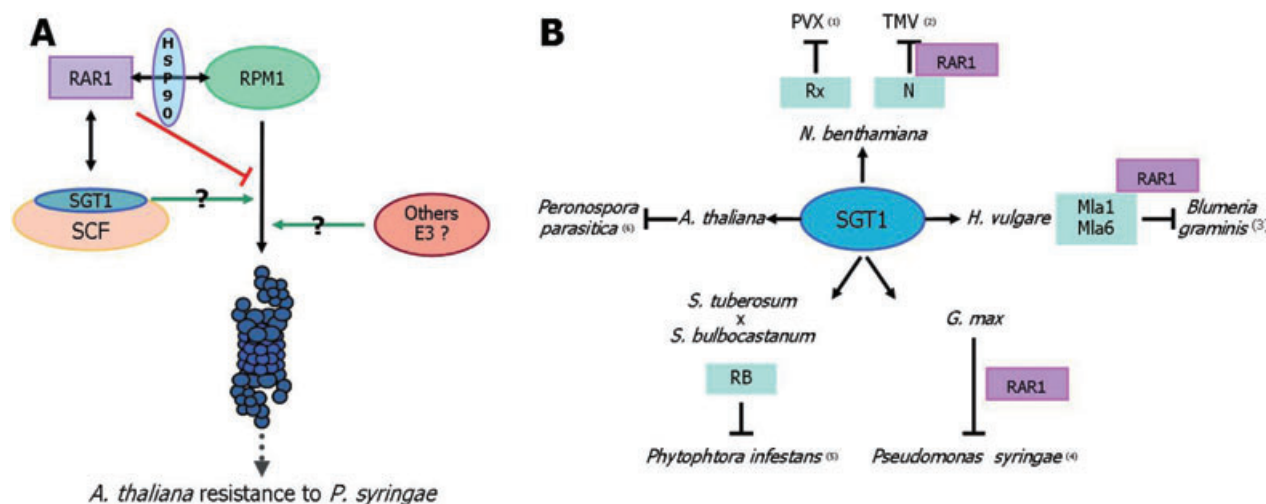


Fig. 2 SGT1 (suppressor of G2 allele of *skp1*) involvement in plant defence mechanisms. (A) RPM1 (resistance to *Pseudomonas syringae* pv. *maculicola* 1) degradation during the onset of the hypersensitive response (HR). To date, the E3 ligase mediating RPM1 degradation still remains unknown. As SGT1 interacts with RAR1 (required for Mla12 resistance 1), a protein that regulates RPM1 stability, SGT1 may be required in this degradation mechanism. (B) Examples of the crucial role played by SGT1 in plant defence mechanisms (*R* gene products are indicated by green shading). PVX, Potato virus X; TMV, Tobacco mosaic virus; (1), Azevedo *et al.*, 2006; (2), Liu *et al.*, 2002; (3), Azevedo *et al.*, 2002; (4), Fu *et al.*, 2009; (5), Bhaskar *et al.*, 2008; (6), Tör *et al.*, 2002.

RAR1 positively regulates *Mla* gene steady-state levels in barley (Bieri *et al.*, 2004). *SGT1* is also implicated in barley disease resistance as its silencing alters powdery mildew resistance (Azevedo *et al.*, 2002). *SGT1*, but not RAR1, is also involved in potato resistance to *Phytophthora infestans* triggered by the *RB* (resistance to *P. infestans-bulbocastanum*1) *R* gene (Bhaskar *et al.*, 2008), whereas the *Nicotiana benthamiana* *SGT1* orthologue (NbSGT1), which interacts with both NbRAR1 and NbSKP1, is required for *N* gene-mediated resistance to TMV (Liu *et al.*, 2002). NbSGT1 silencing causes *N. benthamiana* to lose *R* gene-mediated resistance against Potato virus X (PVX, Potexvirus), *Cladosporium fulvum*, *Phytophthora infestans* and *P. syringae*, and the loss of some (against *P. syringae* pv. *maculicola* or *Xanthomonas axonopodis* pv. *vesicatoria*), but not all [*Cauliflower mosaic virus* (CaMV), *Caulimovirus*; *X. campestris* pv. *campestris*], nonhost resistances, highlighting the role of *SGT1* in the molecular control of a wide range of pathogens (Peart *et al.*, 2002). Furthermore, the silencing of NbSGT1 causes a reduction in the levels of the Rx protein, suggesting a role in the accumulation of some *R* proteins (Azevedo *et al.*, 2006). These studies suggest a crucial role for the ubiquitination pathway in *R* gene-mediated disease resistance, and *SGT1* may be a general factor involved in plant defence mechanisms (Fig. 2B). In agreement with this hypothesis, a recent study has shown that *SGT1* and RAR1 play a role in soybean resistance to *P. syringae*, as they are required for the induction of *R* gene-mediated defence mechanisms, systemic acquired resistance and basal defence (Fu *et al.*, 2009).

Other E3 ubiquitin ligases involved in resistance mechanisms

In addition to the important role of *SGT1*, several examples underline the involvement of other E3 ligases in plant defence mechanisms, regardless of the type of pathogen. Table 1 summarizes some of these examples, highlighting the diversity of targeted pathogens and citing the corresponding substrates (if known).

OsRHC1 (RING HC domain 1), a rice RING zinc finger protein, confers improved resistance to *P. syringae* pv. *tomato* DC3000 to *A. thaliana*. This resistance is proteasome dependent as it is abolished by MG132, a known proteasome inhibitor (Cheung *et al.*, 2007). Overexpression of OsDRF1 (defence-related F-box 1), an F-box protein whose expression is induced by benzothiadiazole, a chemical inducer of plant defence responses, also enhances disease resistance to *P. syringae* pv. *tabaci* and ToMV in transgenic tobacco (Cao *et al.*, 2008). OsDRF1 seems to be implicated only in responses to biotic stresses, as no evidence of OsDRF1 implication in abiotic stress tolerance has been found. In *Solanum lycopersicum* cv. Micro-Tom, the expression of E3 ligase LeATL6 (*Arabidopsis* toxico para levadura-toxic to fungi-6) has also been found to be elicited in roots using a cell wall protein fraction of *Pythium oligandrum*. Overexpression of LeATL6, driven by the CaMV 35S promoter, activates the transcription of some members of the pathogenesis-related (*PR*) gene family in wild-type tomato, but not in *jai1-1* (jasmonate insensitive 1-1) mutants, in which the jasmonate signalling pathway is impaired, suggesting that LeATL6 may play a role in elicitor-induced resistance via a jasmonate-dependent pathway (Hondo *et al.*, 2007).

Table 1 Some plant E3-ligases involved in defence mechanisms.

Protein	Organism	E3 ligase type	Predicted substrates	Pathways	Pathogen	References
ACIF1	<i>N. tabacum</i>	F-box	JAZ proteins	Cf-genes mediated HR SA signalling JA signalling	TMV	(van den Burg <i>et al.</i> , 2008)
ACRE276	<i>N. tabacum</i>	U-box			TMV	(Yang <i>et al.</i> , 2006)
ATL1	<i>S. lycopersicum</i>	RING				(Hondo <i>et al.</i> , 2007)
BAH1/NLA	<i>A. thaliana</i>	RING			<i>P. syringae</i>	(Yaeno & Iba, 2008)
COI1	<i>A. thaliana</i>	F-box				(Devoto <i>et al.</i> , 2002, Thines <i>et al.</i> , 2007)
DRF1	<i>O. sativa</i>	F-box			<i>P. syringae</i> ToMV	(Cao <i>et al.</i> , 2008)
EBF1, EBF2	<i>A. thaliana</i>	F-box	EIN3	ethylene signalling PAMP-triggered resistance (negative regulation)		(Binder <i>et al.</i> , 2007)
PUB22, 23, 24	<i>A. thaliana</i>	U-box				(Trujillo <i>et al.</i> , 2008)
RHC1	<i>O. sativa</i>	RING			<i>P. syringae</i>	(Cheung <i>et al.</i> , 2007)
SGT1	<i>A. thaliana</i>	F-box			<i>P. parasitica</i>	(Austin <i>et al.</i> , 2002, Tör <i>et al.</i> , 2002)
SGT1	<i>A. thaliana</i>	F-box			<i>P. syringae</i>	(Kawamura <i>et al.</i> , 2009)
SGT1	<i>A. thaliana</i>	F-box			<i>R. solanacearum</i>	(Kawamura <i>et al.</i> , 2009)
SGT1	<i>G. max</i>	F-box			<i>P. syringae</i>	(Fu <i>et al.</i> , 2009)
SGT1	<i>N. benthamiana</i>	F-box			TMV	(Liu <i>et al.</i> , 2002)
SGT1	Potato	F-box			<i>P. infestans</i>	(Bhaskar <i>et al.</i> , 2008)
SON1	<i>A. thaliana</i>	F-box			<i>P. parasitica</i>	(Kim & Delaney, 2002)
SPL11	<i>O. sativa</i>	U-box			<i>P. syringae</i> <i>M. grisea</i> <i>X. oryzae</i>	(Zeng <i>et al.</i> , 2004)

The *Nicotiana tabacum* ACIF1 (Avr9/Cf9-INDUCED F-BOX 1) F-box protein, which associates with ASK1 and CUL1 in the SCF complex, is required for elicitor-induced HR and is involved in *N* gene-mediated resistance to TMV (van den Burg *et al.*, 2008). Moreover, the function of *A. thaliana* ACIF1 homologues is linked to abiotic and biotic stress responses by regulating abscisic acid- and jasmonate-responsive genes (van den Burg *et al.*, 2008).

The *Arabidopsis* RING E3 ligase HISTONE MONOUBIQUITINATION 1 (HUB1) is also a regulatory component of plant defence against necrotrophic fungal pathogens via the regulation of gene expression. Indeed, loss-of-function *A. thaliana* *hub1* mutants display extreme susceptibility to *Botrytis cinerea* and *Alternaria brassicicola* (Dhawan *et al.*, 2009). As HUB1 is involved in H2B histone monoubiquitination, which does not lead to degradation by the 26S proteasome but to chromatin regulation and modulation of gene expression, the action of HUB1 might be mediated by a direct effect on gene expression rather than through the UPS pathway (Dhawan *et al.*, 2009).

Involvement of the 26S proteasome in plant defence

Both 26S RP and CP could play a role in defence against pathogens, but the demonstration so far remains indirect. A screen for TMV-induced nuclear proteins in *Capsicum annuum* cv. Bugang has shown that RPN7 expression is increased at both the transcriptional and translational levels during incompatible interaction (TMV-P₀), but not during compatible interaction (TMV-P_{1,2}), and may thus be involved in PCD (Lee *et al.*, 2006). Three

tobacco 20S subunits, α_3 , α_6 and β_1 , are rapidly and specifically induced at the transcriptional level by cryptogin, a fungal elicitor of defence reactions. The expression of these defence-induced (din) subunits (α_3 din, α_6 din and β_1 din) is correlated with the induction of systemic acquired resistance (SAR), an inducible immune response against a broad spectrum of pathogens, and the production of reactive oxygen species (ROS) (Dahan *et al.*, 2001). It has been postulated that these subunits could replace the corresponding constitutive 20S subunits, leading to a newly reassembled 'plant defence proteasome' (Suty *et al.*, 2003), which could be the plant equivalent to the animal immunoproteasome, induced by interferon- γ (Kloetzel, 2004), even though the proteolytic activities of the plant defence proteasome remain unchanged (Suty *et al.*, 2003). The β_1 din subunit may contribute to ROS regulation during plant defence reactions by inhibiting NADPH oxidase at the transcriptional level (Lequeu *et al.*, 2005), but these data need to be confirmed.

The proteasome-mediated degradation of NPR1 (nonexpressor of pathogenesis-related genes 1), a transcription coactivator critical for SAR, has been shown recently to play a dual role in the regulation of plant immunity. Indeed, in noninduced cells (no SAR), the nuclear proteasome-mediated turnover of NPR1 prevents the inappropriate activation of SAR, whereas in SAR-induced cells, phosphorylation of NPR1 facilitates its recruitment to a cullin-3-based ubiquitin ligase and its proteasomal degradation, stimulating NPR1 target gene expression (Spoel *et al.*, 2009). The authors have hypothesized that such a turnover of 'exhausted' phosphorylated NPR1 from the target promoter allows 'fresh' NPR1 to reinitiate the transcription cycle.

The 20S α_5 subunit may also play a crucial role in plant defence. The expression of the α_5 subunit from *Oryza grandiglumis* (OgPAE1) is induced during defence responses, and the overexpression of OgPAE1 in *A. thaliana* leads to resistance against *Bo. cinerea*, but the underlying mechanism(s) remains unknown (Jeon *et al.*, 2008). The implication of the proteasome α_5 subunit in plant defence might be more important in resistance to viruses, as it has been shown to specifically cleave *in vitro* TMV RNA (Ballut *et al.*, 2003; Petit *et al.*, 1997). To date, a key question remains unresolved, that of an *in vivo* contribution to plant antiviral defence for this catalytic RNase activity identified *in vitro*. In such a scenario, plants would have evolved two antiviral mechanisms based on RNA degradation: RNAi and 20S-mediated degradation (Ballut *et al.*, 2005). 20S-mediated RNA degradation could be considered as a first component of plant antiviral defence, targeting nonhost RNAs, a less fine-tuned mechanism than RNAi. Interestingly, calf liver proteasomes hydrolyse HIV-TAR, a tRNA-like structure at the 5'-end of HIV mRNAs (Gautier-Bert *et al.*, 2003). The authors have suggested that this degradation may be part of a defence mechanism, as HIV mRNAs lacking their 5'-end are poorly translated.

Viral proteins themselves are the target of UPS. Indeed, the degradation of virus movement proteins (MPs) by the 26S proteasome seems to be a common event during plant-virus interactions. TMV MP was first shown to be degraded *in vivo* by the 26S proteasome. Proteasome inhibitors lead to an increased stability of this protein, which then accumulates in the endoplasmic reticulum in a polyubiquitinated form (Reichel & Beachy, 2000). The *Turnip yellow mosaic virus* (TYMV, *Tymovirus*) 69K MP is also specifically degraded by the 26S proteasome via a ubiquitin-dependent mechanism (Drugeon & Jupin, 2002). More recently, the PVX TGBp3 (triple gene block protein 3) protein, required for virus cell-to-cell movement, and the *Potato leafroll virus* (PLRV, *Luteovirus*) MP, have also been shown to be degraded by the 26S proteasome pathway (Ju *et al.*, 2008; Vogel *et al.*, 2007). This degradation may be a common feature of viral MPs, regulating virus spread and limiting host cell damage: Gillespie *et al.* (2002) showed that impairment of TMV MP degradation using proteasome inhibitors results in improved viral transport (Gillespie *et al.*, 2002). Interestingly, viral protein degradation may not only play a role in plant defence: viruses may regulate their cycle by targeting abnormal or excess proteins for degradation. Indeed, mutant and denatured, but not wild-type, TMV capsid proteins are massively targeted by the ubiquitin-conjugating pathway in tobacco. As functional proteins are not targeted, this ubiquitination event may not be related to defence mechanisms (Jockusch & Wiegand, 2003). However, whether these degradation events are obligatory steps in the virus cycle to ensure effective invasion, or whether they play a role in defence responses, still remains unknown. Interestingly, about 1/2000 of functional TMV capsid proteins undergo monoubiq-

uitination (Dunigan *et al.*, 1988). Such a modification may play a role, so far unknown, during the TMV cycle.

Ubiquitin conjugation of viral MPs may sometimes more probably reflect a misfolded protein pathway activation than a plant defence mechanism (Jockusch & Wiegand, 2003), supporting the idea that viruses use UPS to control the quality of their own proteins and illustrating the hijacking of this system by pathogens. However, there is now evidence that a number of pathogens have evolved specific systems to inhibit and/or harness UPS to their means by mimicking host proteins to promote their own survival.

SHUTTING OFF THE PROTEASOME CATALYTIC ACTIVITIES

Bacterial proteasome inhibitors

Polyketides and nonribosomal peptides are large classes of natural compounds, including important agrochemical and pharmaceutical products, such as antifungals, antibiotics and antitumour agents (Cane & Walsh, 1999). *Pseudomonas syringae* pv. *syringae* secretes syringolin A (SylA), a peptide derivative synthesized by a mixed nonribosomal peptide/polyketide synthetase. SylA is a virulence factor in the *Phaseolus vulgaris*-*P. syringae* pv. *syringae* interaction, as a SylA-negative mutant strain is less virulent than the wild-type. SylA irreversibly inhibits, both *in vitro* and *in vivo*, all three *A. thaliana* 20S proteolytic activities by covalently binding to the catalytic subunits (Groll *et al.*, 2008). Glidobactin, a molecule structurally related to SylA, is another nonribosomal peptide/polyketide synthetase product isolated from the soil bacterium *Burkholderia cepacia*, which inhibits *in vitro* two of the three 20S catalytic activities (Groll *et al.*, 2008). The gene cluster involved in glidobactin synthesis is also found in *Burkholderia pseudomallei*, the agent causing melioidosis, an important public health disease. As a result of its proteasome inhibitory activity, glidobactin could be involved in *Bu. pseudomallei* virulence (Schellenberg *et al.*, 2007).

Virus inhibition of 26S proteasome activities

LMV affects the 20S proteasome activity: during infection, high-molecular-weight proteasome-containing complexes are found in pea, suggesting that 20S aggregates in complexes that do not exist in healthy plants and may include other molecules of host or viral origin (Ballut *et al.*, 2005). The viral protein HcPro (helper component proteinase), a key potyviral protein involved in various steps of the viral cycle (replication, cell-to-cell movement, aphid transmission) and already known as a suppressor of post-transcriptional gene silencing (PTGS) (Lakatos *et al.*, 2006; Merai *et al.*, 2006; Plisson *et al.*, 2003), associates *in vivo* and *in vitro* with purified 20S proteasome and is probably part of the

Table 2 Some pathogen E3 ligases involved in virulence mechanisms.

Protein	Organism	E3 ligase type	Interacting proteins	Reference
AvrPtoB	<i>Pseudomonas syringae</i>	RING/U-box	Fen FLS2	(Rosebrock <i>et al.</i> , 2007) (Göhre <i>et al.</i> , 2008)
CLINK	<i>Faba necrotic yellows virus</i>	F-box	SKP1	(Aronson <i>et al.</i> , 2000; Lageix <i>et al.</i> , 2007)
GALA1-7	<i>Ralstonia solanacearum</i>	F-box	ASK1 and ASK2	(Angot <i>et al.</i> , 2006; Pouyemiro & Genin, 2009)
HopM1	<i>P. syringae</i>	No known structure	AtMIN proteins	(Nomura <i>et al.</i> , 2006)
P0	<i>Beet western yellows virus, Cucurbit aphid-borne yellows virus</i>	F-box	ASK1, ASK2 and AGO1	(Baumberger <i>et al.</i> , 2007; Bortolamiol <i>et al.</i> , 2007; Pazhouhandeh <i>et al.</i> , 2006)
VirF	<i>Agrobacterium tumefaciens</i>	F-box	VIP1 and VirE2	(Tzfira <i>et al.</i> , 2004)

aggregates observed *in planta*. This hypothesis has recently been reinforced by the demonstration that the *Potato virus Y* (PVY, *Potyvirus*) HcPro physically interacts with three *A. thaliana* proteasome subunits: α_1 , β_2 and β_5 (Jin *et al.*, 2007). Unpublished results from our laboratory indicate, in addition, that the LMV HcPro interacts *in vivo* with the *A. thaliana* α_5 subunit. Furthermore, HcPro has been shown *in vitro* to inhibit the 20S-associated RNase activity, as pre-incubation of cauliflower purified 20S proteasomes with HcPro significantly inhibits the degradation of TMV RNA (Ballut *et al.*, 2005). By contrast, HcPro seems to stimulate slightly 20S chymotrypsin and trypsin-like proteolytic activities (Ballut *et al.*, 2005). To date, the role of such modulations of proteasome enzymatic activities remains unclear, but they represent prime candidates for the harnessing of cellular functions for the virus' own ends or for the targeting of host cell defence mechanisms by the virus.

Although little is known about proteasome inhibition by plant viruses, there are numerous examples of such a strategy for animal viruses, with HIV-1 being a well-studied case. The HIV Tat protein interacts *in vitro* and *in vivo* with the α_3 and α_7 subunits, with six β subunits and with the interferon- γ -inducible β_{2i} and β_{5i} subunits, leading to the inhibition of proteasome proteolytic activities *in vivo* (Apcher *et al.*, 2003). Tat also interferes with immunoproteasome assembly by inhibiting 20S–11S RP complex formation (Seeger *et al.*, 1997). The E1A (early region1A) adenovirus protein also inhibits proteasome activity by interacting with the 19S RP subunits S2, S4 and S8, leading to an increased expression of p53 (Zhang *et al.*, 2004). This may be a way in which adenoviruses regulate the host cell cycle, as p53 controls cell cycle checkpoints (Bertrand *et al.*, 2004).

As shown above, some plant pathogens have evolved strategies to inhibit UPS. However, others reprogram it by using proteins that mimic host UPS proteins. This trick enables pathogens to circumvent the host's defence responses or to harness the host UPS for their own purposes.

UPS USURPATION BY PATHOGENS

There is growing evidence that pathogens hijack the host UPS and that these mechanisms are potentially involved in virulence.

Table 2 summarizes some of these examples, including the corresponding cellular targets (if known).

Bacterial usurpation of the host UPS

There are numerous indications that bacterial pathogens are able to harness or reprogram their host's UPS. The type III and type IV secretion systems (T3SS and T4SS) are macromolecular protein complexes of major importance for the virulence of pathogenic bacteria infecting either plants or animals. They allow pathogens to inject type III- or type IV-secreted effectors (T3SE or T4SE) directly from the bacterial cell into the host cell cytoplasm. T3SE and T4SE are often the primary weapons used to target host defence signalling (McCann & Guttman, 2008; Juhas *et al.*, 2008). During host transformation, *Agrobacterium* exports to the host cell, via its T4SS, a T-DNA–protein complex composed of a single-stranded T-DNA packaged by several VirE2 proteins and a single VirD2 protein (Tzfira & Citovsky, 2002). VirE2 interacts with *A. thaliana* VIP1 (VirE2 interacting protein 1) protein, which facilitates the nuclear uptake of VirE2 and thus may participate in the nuclear import of the entire T-DNA complex (Tzfira *et al.*, 2001). Before integration in the host genome, the T-DNA must be uncoated by a process that is so far unknown. However, VirF, another exported bacterial protein, may be involved in T-DNA uncoating as it contains an F-box motif domain and interacts with *A. thaliana* ASK1 and ASK2 *in vitro*. VirF may therefore be incorporated into an SCF complex including host components (Schrammeijer *et al.*, 2001). VirF interacts *in planta* with VIP1, leading to its intranuclear destabilization via targeted proteasome degradation. The observation that 26S proteasome inhibitors block T-DNA–protein complex uncoating (Tzfira *et al.*, 2004) led to the proposition of a model in which VirE2 degradation by the host cell UPS would be a consequence of the ASK1/2–VirF–VIP1–VirE2 network of interactions.

Some T3SE proteins act as molecular mimics of eukaryotic proteins and target essential cell mechanisms. *Ralstonia solanacearum* encodes seven GALA (GAXALA domain proteins) T3SEs which contain F-box domains (Angot *et al.*, 2006). In a two-hybrid system, GALA1, GALA5, GALA6 and GALA7 interact physically with *A. thaliana* ASK1 and ASK2. Furthermore, a

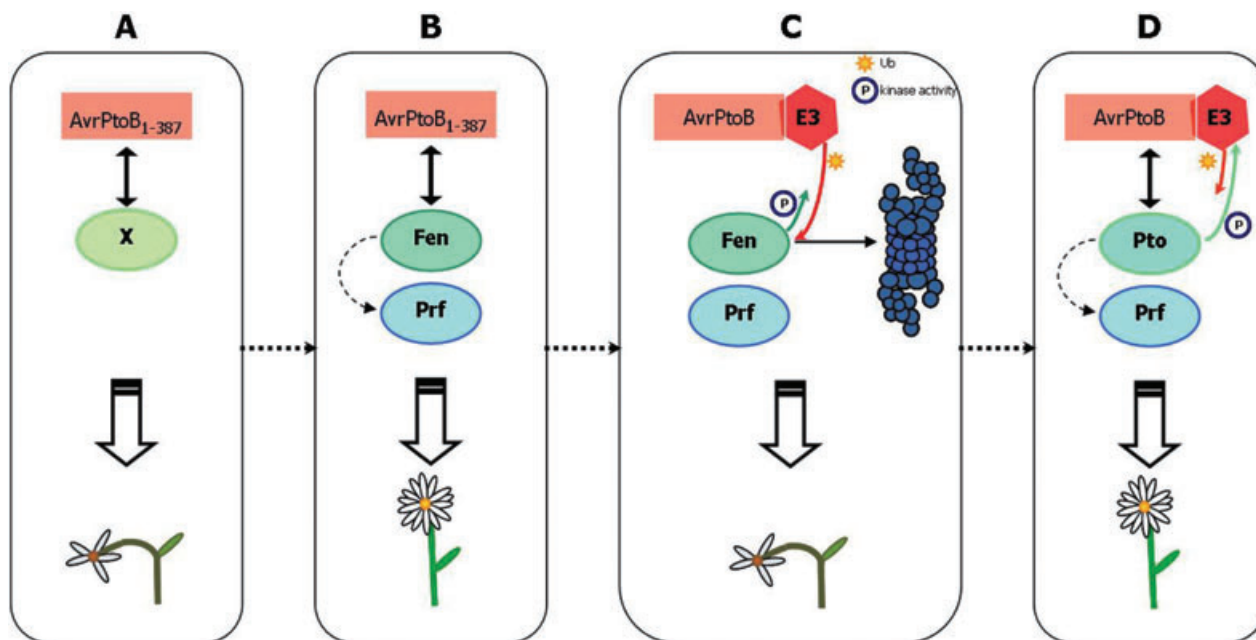


Fig. 3 Evolution of the *Pseudomonas syringae* virulence factor AvrPtoB and the host R proteins Fen (fenthion sensitivity) and Pto. (A) AvrPtoB_{1–387} overcomes plant basal defences, causing disease. (B) Fen recognizes AvrPtoB_{1–387} and triggers programmed cell death (PCD)-based plant resistance. (C) AvrPtoB acquires an E3 ligase domain, causing ubiquitin/26S proteasome system (UPS)-dependent Fen degradation, thus enhancing disease. (D) Pto, impervious to E3 ligase activity (via its kinase activity and phosphorylation of AvrPtoB), evolves to recognize AvrPtoB, restoring plant immunity. Adapted from Rosebrock *et al.*, 2007.

GALA7 knockout (KO) mutant strain shows a dramatic reduction in virulence on *Medicago truncatula*, but not on *A. thaliana* or tomato. Simultaneous deletion of all seven GALA genes leads to a loss of virulence of *R. solanacearum* on *A. thaliana* and tomato. These proteins thus seem to be an essential, if partially redundant, virulence factor acting via an SCF-dependent mechanism, where GALA effectors are involved as F-box-containing proteins to interfere with the host UPS (Angot *et al.*, 2006). The host proteins targeted for degradation by SCF^{GALA} have yet to be identified, but could be crucial elements of the plant defence response (Pouymiro & Genin, 2009).

Another well-studied case of T3SE mimicking and directly interfering with the host UPS is the *P. syringae* pv. *tomato* AvrPtoB. This small triple-helix protein is targeted to the plasma membrane and recognized by the Pto R protein, mediating HR-based PCD in resistant plants via the Prf (*Pseudomonas* resistance and fenthion sensitivity) protein (Mucyn *et al.*, 2006). The AvrPtoB C-terminal region shows homology to RING-finger and U-box E3 proteins (Janjusevic *et al.*, 2006; Zipfel & Rathjen, 2008). Mutations within this domain result in reduced virulence of the bacterium *in vivo* (Janjusevic *et al.*, 2006). As *P. syringae* does not have an endogenous UPS, this observation suggests that this bacterium has acquired an E3 domain protein, allowing it to harness the host UPS to target host proteins for degradation and thus a gain in virulence (Abramovitch *et al.*, 2006; Block *et al.*, 2008). AvrPtoB addresses the host R protein Fen (fenthion

sensitivity), a serine/threonine kinase, for proteasomal degradation, as proteasome inhibitors cause Fen accumulation when expressed with full-length AvrPtoB, but not with truncated AvrPtoB_{1–387} lacking the C-terminal E3 domain (Rosebrock *et al.*, 2007). Interestingly, Fen recognizes truncated AvrPtoB_{1–387}, leading to PCD-mediated resistance in Pto-lacking tomato varieties via the activation of Prf-mediated immunity (Rosebrock *et al.*, 2007). This complex relationship illustrates the co-evolution between pathogen virulence and plant R proteins, and how the plant UPS can be exploited by pathogens to promote their virulence (Fig. 3) (Craig *et al.*, 2009; Rosebrock *et al.*, 2007). In such a co-evolution scenario, several steps are envisioned. First, the pathogen AvrPtoB_{1–387} evolves to overcome plant basal defences. The plant Fen kinase then evolves to recognize AvrPtoB_{1–387}, triggering a PCD-mediated resistance. Next, *P. syringae* 'incorporates' an E3 domain into AvrPtoB, targeting Fen for degradation. Finally, the Pto kinase evolves to recognize AvrPtoB and to be impervious to AvrPtoB ubiquitination activity, thus restoring plant immunity through PCD-based mechanisms (Delaure *et al.*, 2008; Rosebrock *et al.*, 2007). Pto polyubiquitination avoidance has recently been elucidated: Pto, via its kinase activity, phosphorylates AvrPtoB, inactivating its E3 domain and thus triggering plant defence mechanisms (Ntoukakis *et al.*, 2009). Interestingly, AvrPtoB also targets the *A. thaliana* FLS2 (flagellin sensing 2) protein for proteasomal degradation, impairing PAMP perception and subsequent plant defence mechanisms

(Göhre *et al.*, 2008). AvrPtoB-mediated degradation of host proteins involved in different defence steps thus seems to be a crucial mechanism for *P. syringae* virulence.

HopM1 is another *P. syringae* virulence protein translocated into the host cell via the T3SS. Using transgenic plants, it has been shown that HopM1 constructs lacking the C-terminal region interact with 21 *A. thaliana* AtMIN (*A. thaliana* HopM1 interactors) proteins, but no interactors are found when using full-length HopM1 (Nomura *et al.*, 2006). HopM1-dependent destabilization of AtMIN proteins is not affected by protease inhibitors, but is blocked by proteasome inhibitors, leading to the accumulation of polyubiquitinated proteins. Among AtMIN proteins, AtMIN7 encodes one of the eight members of the adenosine diphosphate (ADP) ribosylation factor (ARF) guanine nucleotide exchange factor (GEF) protein family, key components of vesicle trafficking that may play a role in the plant immune system by triggering callose deposition on the plant cell wall. *Pseudomonas syringae* may thus have evolved a mechanism to suppress cell wall-associated host defence mechanisms, facilitating bacterial infection (Nomura *et al.*, 2006).

Hijacking of the host UPS by plant viruses

The *Faba bean necrotic yellows virus* (FBNYV, *Nanovirus*), a single-stranded DNA virus, encodes a small protein, CLINK (cell cycle LINK), harbouring an F-box domain, which interacts with a *Medicago sativa* SKP1 homologue both *in vitro* and *in vivo* (Aronson *et al.*, 2000), and may deregulate the host cell cycle by targeting an *A. thaliana* retinoblastoma-related protein (pRB) for degradation (Lageix *et al.*, 2007). The *Beet severe curly top virus* (BSCTV, *Geminivirus*) encodes a C4 protein that enhances host cell division to which viral replication is coupled. *Arabidopsis thaliana* E3 RKP (related to KPC1) protein expression is induced by the BSCTV C4 protein, and *A. thaliana* *rkp* mutants are less susceptible to BSCTV infection (Lai *et al.*, 2009). As RKP has been shown to act as a cell cycle regulator in *Arabidopsis*, these results suggest that this E3 ligase may affect geminivirus replication by the regulation of the host cell cycle (Lai *et al.*, 2009).

Plant viruses are well known as potent inducers and targets of RNAi, as replicating viruses generate double-stranded RNA (dsRNA) (Hannon, 2002). dsRNAs are first cleaved into double-stranded short-interfering RNA (siRNA, 21–23 nucleotides long) by DICER, a specific type III RNase (Ketjing, 2006; ur-Rahman *et al.*, 2008). siRNAs are then incorporated into RISC, a macromolecular complex that destroys target mRNA. The RISC complex contains a member of the Argonaute (AGO) gene family, which binds to siRNA and is implicated in small RNA-mediated regulatory mechanisms (Hannon, 2002; Vaucheret, 2008; Voinnet, 2008). Two of the 10 *A. thaliana* AGO proteins, AGO1 and AGO4, have been shown to be implicated in plant defence mechanisms. *ago4* mutants show enhanced susceptibility to virulent and

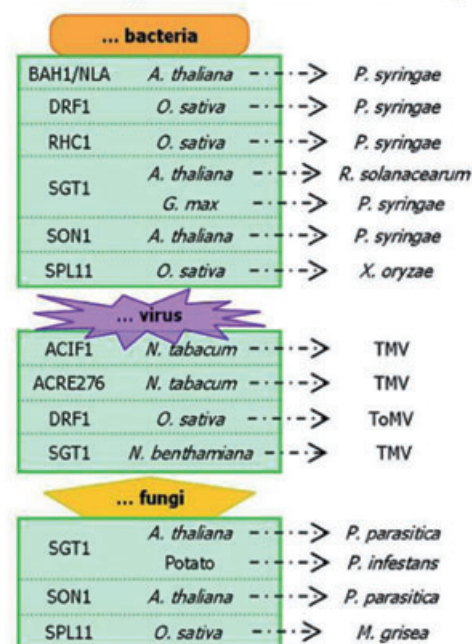
avirulent *P. syringae* pv. *tomato* DC3000 strains, suggesting a role for AGO4 (involved in RNA-directed RNA methylation, RdRM) in the limitation of the bacterial spread in the host (Agorio & Vera, 2007). Interestingly, AGO4 acts independently of RdRM, as mutants altered in components upstream/downstream of AGO4 in RdRM do not show compromised resistance to *P. syringae* (Agorio & Vera, 2007). Hypomorphic *ago1* mutants show increased susceptibility to *Cucumber mosaic virus* (CMV, *Cucumovirus*) (Morel *et al.*, 2002), confirming the essential role played by the RISC complex in plant antiviral immunity. To counteract this resistance mechanism, viruses encode silencing suppressor proteins. To date, more than 35 suppressor proteins have been identified in plant viruses, including the potyviral HcPro (Ding & Voinnet, 2007; Lakatos *et al.*, 2006; Merai *et al.*, 2006). Some of these suppressors interact with the UPS, such as the *Beet western yellows virus* and *Cucurbit aphid-borne yellows virus* (BWYV and CABYV, *Polevirus*) P0 proteins. P0 is a potent silencing suppressor, containing a minimal N-terminal F-box-like domain that enables it to interact *in vivo* with *A. thaliana* ASK1 and ASK2, leading to CUL1-containing complex formation (Pazhouhandeh *et al.*, 2006). P0 F-box domain-lacking viruses are hypovirulent, and recombinant PVX expressing P0 shows more severe symptoms in *N. benthamiana* when compared with the mild wild-type PVX. P0 thus seems to be involved in virus pathogenicity through its F-box domain (Pazhouhandeh *et al.*, 2006). Recently, it has been shown that P0 targets AGO1 for degradation, thus impairing PTGS (Baumberger *et al.*, 2007; Bortolamiol *et al.*, 2007), but the underlying mechanisms still remain unclear, as proteasomal degradation may not be involved (Baumberger *et al.*, 2007).

CONCLUDING REMARKS

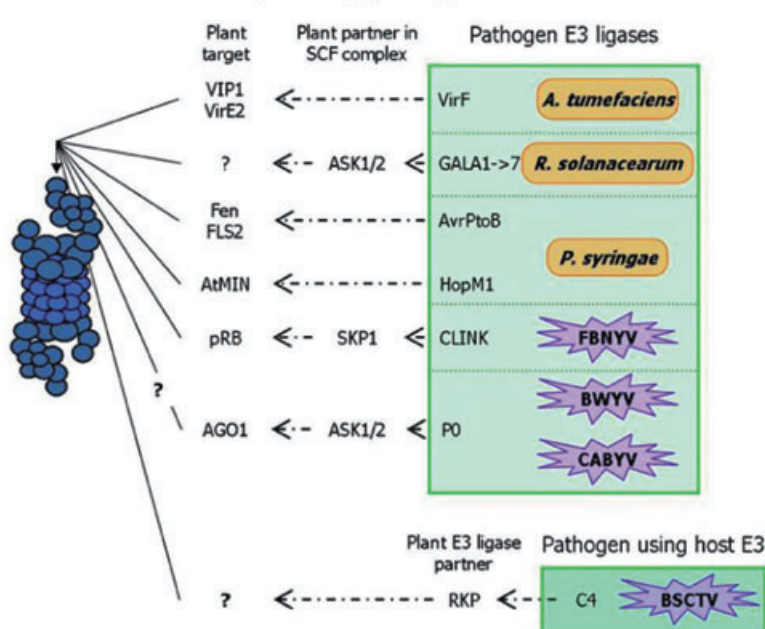
In the past few years, UPS has emerged as an essential protagonist in plant–pathogen interactions. Ubiquitination and subsequent protein degradation seem to occur at several levels of the plant defence mechanism, from basal responses to *R* gene-mediated resistance (Fig. 4). Ubiquitin-dependent protein degradation is known to regulate a wide range of key cellular processes; it is thus not surprising that crucial plant defence elements are found to be UPS substrates. In this respect, it is noteworthy that protein ubiquitination not only triggers degradation, but has also been shown to potentially modify protein localization or function (Deng *et al.*, 2000; Manzano *et al.*, 2008; Schnell & Hicke, 2003). Although not demonstrated so far, these modifications may also play an important role in plant–pathogen interactions.

Protein degradation and/or modifications do not seem to be the only way in which UPS is involved in plant defence. The 20S RNase activity demonstrated *in vitro*, which specifically targets viral substrates, and the inhibitory role of the potyviral protein

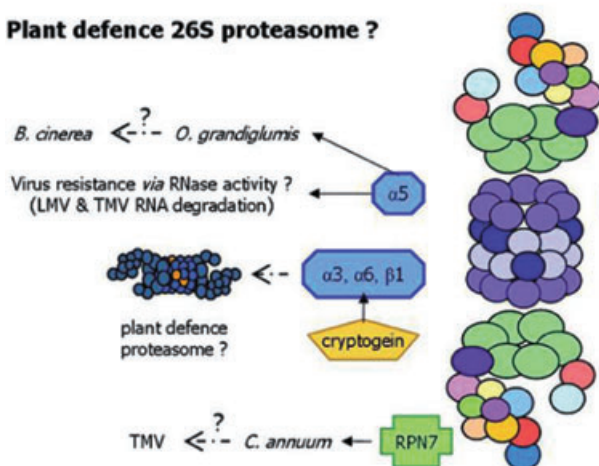
Plant E3 ligases in defence reactions against...



UPS usurpation by pathogens



Plant defence 26S proteasome ?



26S inhibition by pathogens

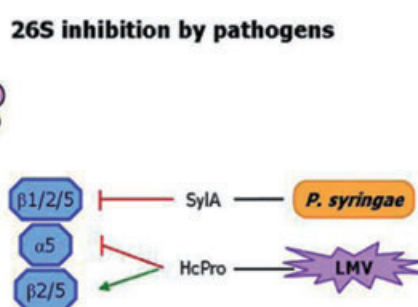


Fig. 4 The ubiquitin/26S proteasome system (UPS), a central element in plant defence and pathogen virulence mechanisms. A summary figure. BSCTV, *Beet severe curly top virus*; BWYV, *Beet western yellows virus*; CABYV, *Cucurbit aphid-borne yellows virus*; FBNYV, *Faba bean necrotic yellows virus*; LMV, *Lettuce mosaic virus*; TMV, *Tobacco mosaic virus*; ToMV, *Tomato mosaic virus*.

HcPro, which interacts with at least four 20S subunits, suggest that both catalytic activities (RNase and protease) harboured by the 20S proteasome could be part of a new antiviral defence pathway. These findings clearly require deeper investigations, but may represent another exciting role for the UPS in plant defence.

UPS is, in many cases, targeted and/or used by pathogens that have evolved to inhibit or interfere with its normal functioning (Kisselev, 2008). The finding that pathogens use E3 ligases for their own purposes provides new insights into the complex molecular interactions between host and pathogen (Fig. 4). The

identification and characterization of the host substrates will show how important is this mimicry tactic in blocking pathogen recognition and/or the activation of defence mechanisms (Angot *et al.*, 2007).

UPS inhibition by pathogens does not only represent an agro-nomical and economical challenge. It may be considered as a chance for human health. Any alteration of UPS components may have disastrous consequences in eukaryotic cells: proteasomal dysfunction is involved in many human diseases, such as cancers, neurodegenerative and autoimmune diseases and

cardiac dysfunction (Dahlmann, 2007). *Pseudomonas syringae* pv. *syringae* SylA irreversibly inhibits the three 20S proteolytic activities (Groll *et al.*, 2008), induces apoptosis and inhibits the proliferation of neuroblastoma and ovarian cancer cells, suggesting that derivatives of this natural compound may serve for the development of novel anticancer drugs (Coleman *et al.*, 2006). Furthermore, proteasome inhibitors also sensitize cancer cells to chemotherapy: proteasome inhibitors trigger oxidative damage, enhancing the chemotherapeutic efficiency in human leukaemia cells (Dasmahapatra *et al.*, 2006; Lü *et al.*, 2008). Such results may thus be a chance: why not use pathogen inhibitors to counteract UPS dysfunction? Proteasome inhibitors may also be used against parasite development. Recently, novel microbial compounds have been identified as proteasome inhibitors: marine *Salinispora tropica* salinosporamide A inhibits 20S activity *in vitro* and protects mice against *Plasmodium* infection (Prudhomme *et al.*, 2008). Some of the newly discovered plant-pathogen proteasome inhibitors may thus lead to novel therapeutic applications in the next few years.

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